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THE SPONTANEOUS ACTIVITY AND PASSIVE AVOIDANCE BEHAVIOR OF RATS EXPOSED BY INHALATION TO BRASS DUST (1 and 10 mg/m^3)

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RESEARCH DIRECTORATE

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Male and female rats were exposed by inhalation to brass dust at 0, 1 or 10 mg/m^3, 6 hr/day, 5 days/wk for either 30 or 65 exposure days. At 14 days post exposure, the 30-day and one 65-day exposure duration group were behaviorally evaluated; a second 65-day group was evaluated 47 days postexposure. There were no statistically significant dose-response or exposure duration effects on passive avoidance (step down) behavior or spontaneous activity. There was no evidence of any direct CNS effect in the rat related to the inhalation of brass dust. There was some evidence of residual recovery effects from exposure-related hyperpnea in the session SA of male rats. Keywords:

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PREFACE

The work described in this report was authorized under Project No. 1L162622A554E, Smoke Toxicology. This work was started in August 1981 and completed in November 1981. The experimental data are recorded in laboratory notebooks 810028 and 810029.

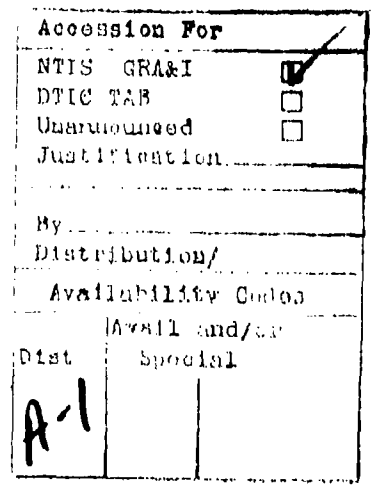
The use of trade names or manufacturers' names in this report does not constitute an official endorsement of any commercial products. This report may not be cited for purposes of advertisement.

In conducting the work described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals" as promulgated by the Committee on Revision of the Guide for Laboratory Animals Facilities and Care of the Institute of Laboratory Animal Resources, National Research Council.

This report has been approved for release to the public.

Acknowledgments

The author wishes to thank Dr. Sandra Thomson, the Principal Investigator, for her cooperation in organizing and scheduling the exposure of these animals during her experiments, and also C. Crouse and D. Burnett for their expertise in setting up and maintaining the exposures.



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CONTENTS

LIST OF FIGURES

LIST OF TABLES

THE SPONTANEOUS ACTIVITY AND PASSIVE AVOIDANCE BEHAVIOR OF RATS EXPOSED BY INHALATION TO BRASS DUST (1 and 10 mg/m^3)

1. INTRODUCTION

This report describes one part of a multilevel assessment of the subchronic toxicity of inhaled brass dust in rodents at 1 and 10 mg/m^3. The effects of inhaling these concentrations for 6 and 13 wk (30 and 65 calendar days, respectively) on blood chemistry, hematology, respiratory physiology, bronchopulmonary lavage fluid, organ/body weight ratios, histopathology and fetal development in the rat, have already been reported.[1,2] Rats utilized for assessing the effects of this compound on pulmonary function, described in Thomson et al.,[1] were also evaluated behaviorally. These behavior results are the subject of this report.

An earlier acute inhalation study in rats employing a higher concentration (100 mg/m^3) for shorter exposure periods indicated that brass dust was essentially a particulate that accumulated primarily in the lung.[3] Its composition[4] suggested that its subchronic toxicity would be related primarily to pulmonary irritation. The results of pulmonary function tests and histopathological examination of rats exposed to brass dust at 1 and 10 mg/m^3 largely supported that expectation.[1] However, without regard to the suspected target organ or resulting biomolecular effect, behavioral testing of rats from this study was designed to evaluate the overall impact such an exposure would have on whole animal behavior. Two simple, naive animal tests were selected for this purpose: the Spontaneous Activity Test (SAT) and the Passive Avoidance Test (PAT).[5] The baseline spontaneous activity (SA) employed is a complex of many discrete behaviors (e.g., walking, rearing, preening, etc.) that appear or disappear in time at differential rates and magnitudes, and are characteristically emitted by an animal when exploring a novel environment.[6] The frequency of the collective activities is initially high but diminishes over time to a very low level as the animal becomes increasingly familiar with its environment. The systematically changing response output of this non-reinforced behavior is the result of two different and competing processes[7] called sensitization (initial facilitation) and habituation (subsequent decline). Because spontaneous activity can be responsive to both peripheral and central stimuli (or drugs), it is useful for detecting a wide variety of toxic

chemical effects, especially when environmental stimuli are carefully controlled.[8,9]

In contrast, the passive avoidance response is a schedule-controlled behavior in which the animals' natural tendency to explore is suppressed by shock (inhibition of sensitization and/or habituation), that is, the animal must find and then learn to remain in a particular corner (the "safe" or correct corner) of the test environment to avoid being shocked (hence the term passive avoidance). The frequency at which the test subject leaves the safe corner during a test session, after having remained in it for a predefined time period, is a measure of the functional integrity of the central nervous system (CNS) as reflected by the rate of Passive Avoidance learning.

2. METHODS AND MATERIALS

2.1 General.

Fifty-four male and fifty-four female Fischer 344 rats were randomly sorted into three large exposure duration groups, each composed of 18 males and 18 females [one 30-day (30A) and two 65-day groups (65A and 65B)]. Each of these groups was further separated into three treatment groups of 12 rats each (50/50 male and female) and randomly assigned one of three treatment conditions: air exposure (control), 1 mg brass dust/m^3, or 10 mg brass dust/m^3 exposure. Inhalation exposures were carried out in three 3000-L chambers simultaneously, 6 hr/day, 5 days/wk until exposure duration conditions were met. At the appropriate time, postexposure ($\geq$72 hr for the 30A and 65A groups and $\geq$1 month for the 65B group), pulmonary function measurements were made on all rats, followed subsequently by behavioral testing. Table 1 is a brief summary of exposure conditions and the postexposure behavioral testing schedule. Details of animal care during the exposure phase of the study (exposure conditions, daily chamber concentrations of brass dust, etc.) appear in the report by Thomson et al.[1]

Twenty-four hours prior to behavioral assessment, the rats to be tested were transferred to clean, sterilized polycarbonate cages (1 rat per cage), containing hardwood chip bedding and stainless steel wire tops, and then moved into the room containing the test environments. The rats were deprived of food during this period but allowed water (bottles) ad libitum. Test room lights were on 24 hr a day, and the ambient temperature was maintained at 72 $\pm$ 2 ^{o}F.

Table 1. Summary of Exposure Conditions and Behavioral Test Schedule for Rats Exposed by Inhalation to Brass Dust.

Exposure Conditions	*Average Air Concentration Brass Dust mg/m3 ± SD	CTs	Treatment mg/m^3	Number of Rats M	F	Behavioral Testing: Calendar Days Post-Exposure
30 Day	0	0	0	6	6	14
	0.97 ± 0.36	10,440	1	6	6	14
	9.50 ± 2.35	102,542	10	6	5**	14
65 Day (A)	0	0	0	6	6	14
	0.94 ± 0.30	22,061	1	6	6	14
	9.05 ± 2.18	211,609	10	6	6	14
65 Day (B)	0	0	0	5**	6	47
	0.94 ± 0.30	22,061	1	6	6	47
	9.05 ± 2.18	211,609	10	6	6	47

*Adapted from CRDEC-TR-84027.
**One rat died prior to testing.

2.2 Behavioral Tests.

2.2.1 SAT.

Aggregate spontaneous activity[10] was measured using a capacitance type device, the Stoelting Electronic Activity Monitor (EAM). The EAM was configured with three activity sensors each containing a 13 in. by 9 in. by 10 in. Plexiglass test environment. All three sensor units were housed in a single, ventilated, unlit (light tight), and sound retardant enclosure. During testing, a 50 db white noise was broadcast in the enclosure via three PM speakers mounted adjacent to each environment. Movement within an environment induced a small voltage in the sensor plate roughly proportional to the magnitude of movement. Two adjustable activity detectors were connected to each sensor; one was calibrated to pick up and convert (activity response, R) each discrete movement with a magnitude greater than or equal to tremors (L1 = total Rs) to a pulse. The other was calibrated to pick up and convert each discrete movement with a magnitude greater than or equal to locomotion (L2 = gross Rs). These responses were automatically collected on-line (BRS/LVE Interact System) and printed out for each rat at 1-min intervals

throughout a test session (24 min). Fine Rs per min, the difference between total and gross Rs (L1 - L2), were also calculated and recorded.

Gross, fine, and total Rs were evaluated in terms of treatment effects on (1) cumulative session Rs, (2) sensitization (highest number of responses per minute), (3) time to sensitization (number of 1-min intervals up to and including sensitization), and (4) the rate of habituation (slope of the declining Rs/min during a test session).

2.2.2 PAT.

Three test environments (12 in. by 13 in. by 14 in.), operationally analogous to the "step down" environment described by Meyers,[11] were individually enclosed within ventilated sound retardant boxes and used to condition passive avoidance behavior. Each environment contained (1) a 2-1/2 watt, flush mounted ceiling light (on only during a test session), (2) a shock grid floor consisting of 18 parallel and electrically isolated stainless steel rods (3/16 in. dia), spaced 1/2 in. apart, and (3) a pair of photosensor units (BRS/LVE Company) in each corner. The units were mounted so that their beams intersected within the environment at a point 1-1/2 in. above the grid floor and 2 in. from both walls forming a corner.

The grid rods were connected by cable to a shock generator/scrambler (Grason Stadler Model E1064GS). When activated by a programmed pulse, the generator delivered 350 volts rms to the grid floor at 0.2 ma for 0.5 sec. Whenever one or both of the photocell beams in the safe corner (left front corner) were uninterrupted, the shock circuit was activated every 5 sec; whenever both beams were simultaneously interrupted, the shock circuit was deactivated.

The basic data collected during a 24-min test session were (1) total passive avoidance responses (PARs) which are the number of times the shock circuit was continuously interrupted for 5 sec or more, (2) total number of shocks delivered, and (3) total time both beams in the safe corner were not simultaneously interrupted (shock time).

2.2.3 Testing Procedures.

Behavioral assessment of all 36 rats from a single exposure duration group was completed on the day testing began.

One rat from each of the three treatment groups was tested simultaneously, first in the SAT and then in the PAT. Individual body weights were determined and recorded about 40 min prior to the SAT. In order to minimize any effects on behavior that might arise from minor differences between test environments, a random block design was used, that is, an equal number of rats from each treatment group (2 males and 2 females) were evaluated in each of the three SAT and PAT environments, respectively.

In an effort to minimize scent related behavioral interactions that could arise from the random testing of male and female rats in the same apparatus, all male rats were tested first, after which the apparatus was thoroughly cleaned and dried before the females were tested. Cleaning involved washing each environment with a mild detergent solution, rinsing it with water, and then rinsing with 70% alcohol.

2.2.4 Statistical Analysis.

Statistical comparisons were made only between the treatment groups within each exposure duration group. Differences between treatment group body weights, as well as the cumulative session data from the SAT and PAT were evaluated by the appropriate analysis of variance (ANOVA)[12] [SAT data by a random block, 3 by 2 by 2 factorial ANOVA (i.e., treatment, sex, and activity level), the PAT and body weight data by complete random one-way ANOVAs of each parameter for each sex]. A complete random 3 by 2 by 2 factorial ANOVA (treatment, sex and activity level) was employed to evaluate differences in the magnitude of sensitization. In all cases where ANOVA F ratios indicated a significant difference in variance, significantly different means were identified using Tukey's HSD test.[12] Differences in the time to sensitization were evaluated by doing Chi Square analyses of the number of 1-min intervals up to and including the interval of peak activity.

Habituation of spontaneous activity was assessed by first doing a linear regression analysis of the session data generated at 1-min intervals and statistically comparing the appropriate slopes[12] for each sex.

3. RESULTS

3.1 SAT.

Figures 1 and 2 are session plots for male and female rats respectively, of mean total, gross, and fine activity responses per min, generated by each treatment group after

30 days of inhalation exposure. Identical plots for the 65A and 65B duration groups appear in Figures 3 and 4 and Figures 5 and 6, respectively. These plots clearly demonstrate the early development of response sensitization followed by a variable but gradual habituation of spontaneous activity through the end of each test session.

The amount of response sensitization and the time to its development appear in Table 2. Analysis of these data indicate there were no statistically significant effects of either brass dust exposure concentration or duration of exposure on response sensitization parameters generated by either male or female rats.

Table 3 lists the response habituation slope ($\pm$95% C.L.) and corresponding coefficients of determination (r^2), for each exposure duration group, by sex and treatment. The rate (slope) of response habituation among male rats was significantly different (greater) from the control value only among those males in the 65B duration group previously exposed to 1 mg brass dust/m^3. Although there were no postexposure (65B) rate differences in the female rats, there were some significant increases in response habituation after 30 days exposure and significant decreases after 65A days exposure. However, in each comparison involving a significant difference, except one, the low r^2 of one or both regression lines indicated non-linearity of the data, making the significance of these differences highly questionable.

In addition, there were no statistically significant treatment effects on cumulative SA (total, gross, or fine activity) of male or female rats in any exposure duration group (Table 4). There were, however, in males only, consistent dose-related reductions in all three measures of SA in the 30A and 65A duration groups. Post exposure (65B) males exposed to brass dust at 1 mg/m^3 exhibited a greater increase in SA than those exposed at 10 mg/m^3.

3.2 PAT.

The PAT data are summarized in Table 4. Compared to controls, there were no significant differences in mean passive avoidance responses (PARs), emitted by either sex or group exposed to brass dust, from any of the duration groups. The only significant effects of brass dust exposure on passive avoidance behavior was seen in the 30A females, where an increase was

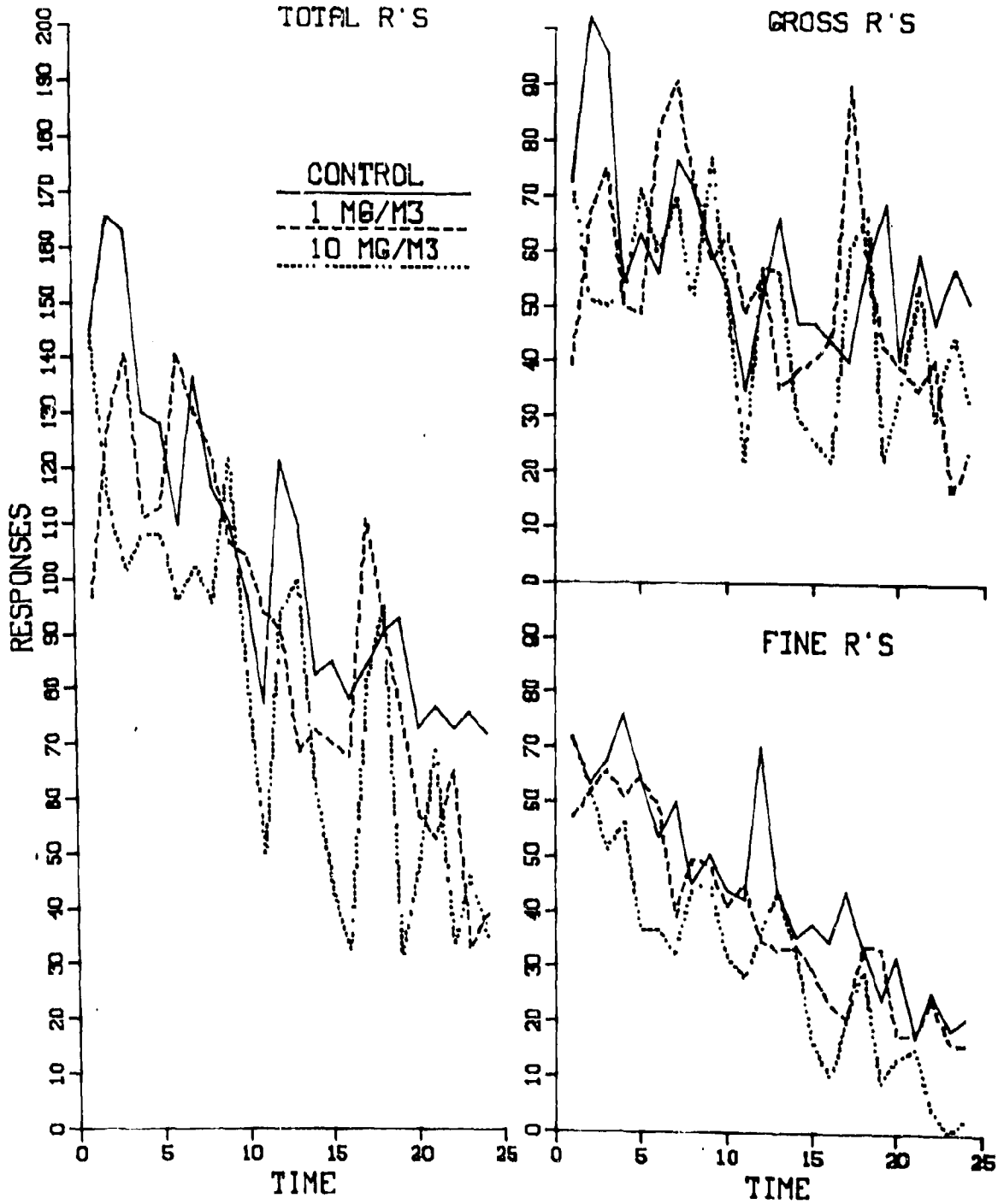

Figure 1. Male Rats, Spontaneous Activity Responses (Rs) Per Min, 30 Day (A) Brass Dust Exposure.

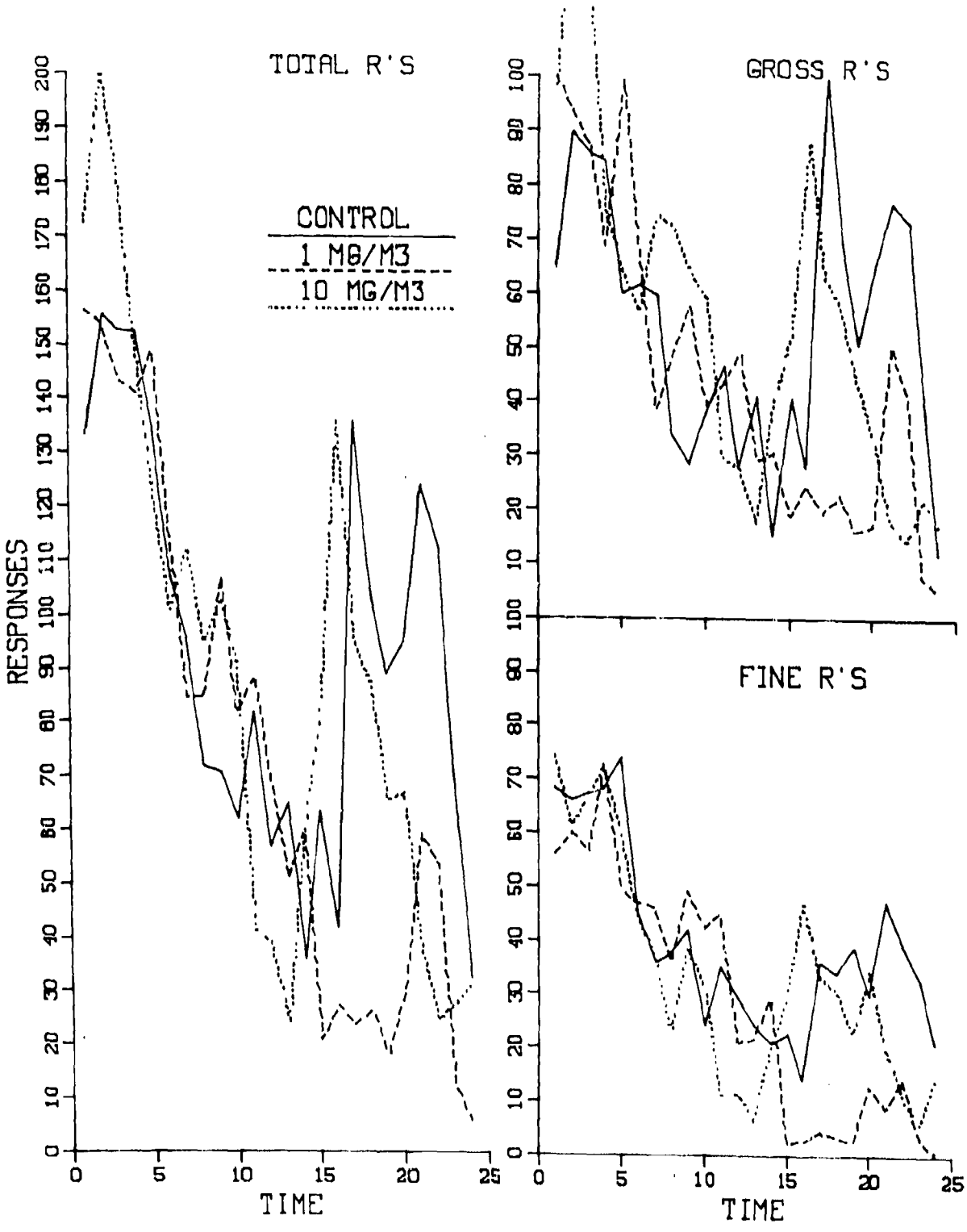

Figure 2. Female Rats, Spontaneous Activity Responses (Rs) Per Min, 30 Day (A) Brass Dust Exposure.

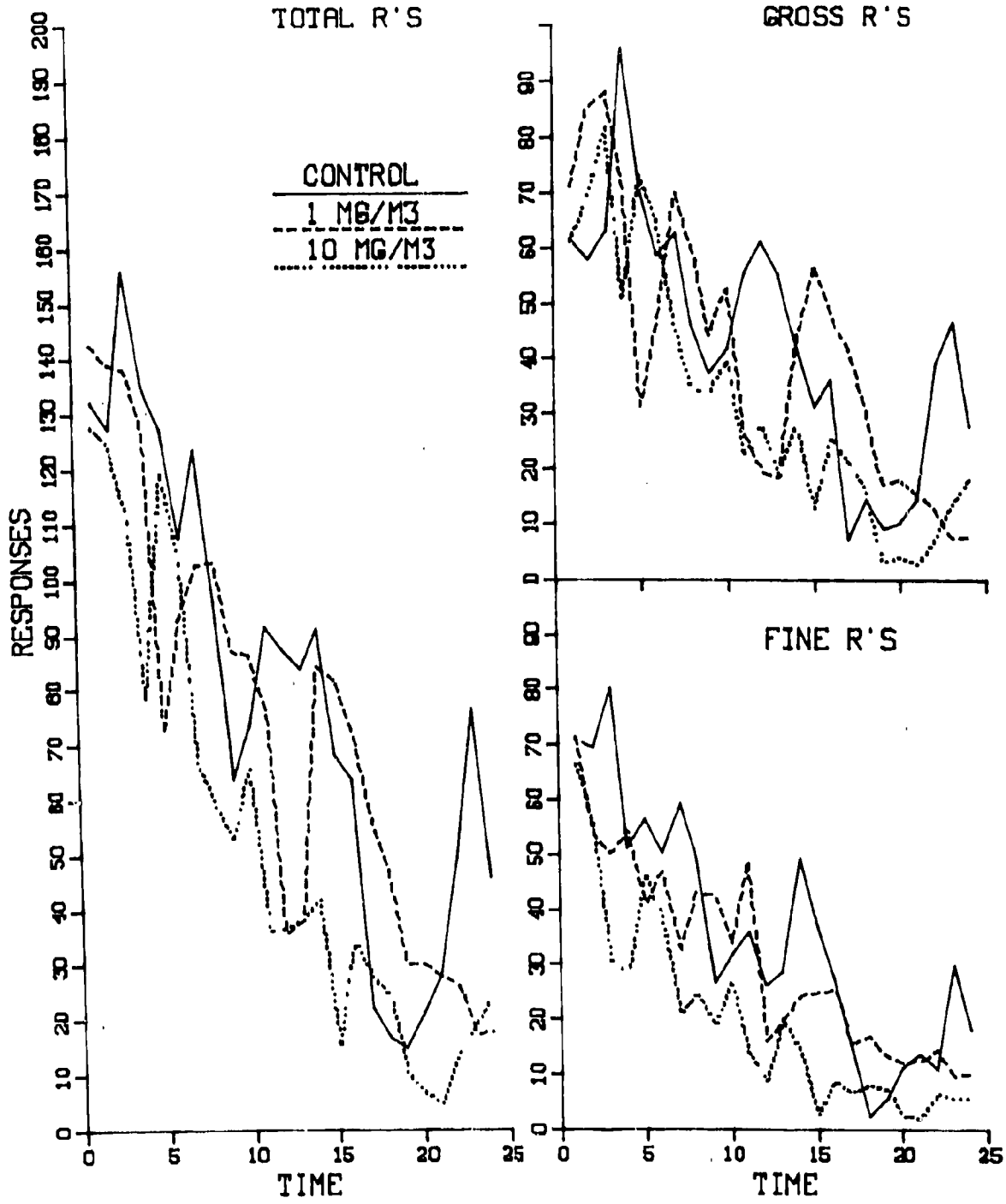

Figure 3. Male Rats, Spontaneous Activity Responses (Rs) Per Min, 65 Day (A) Brass Dust Exposure.

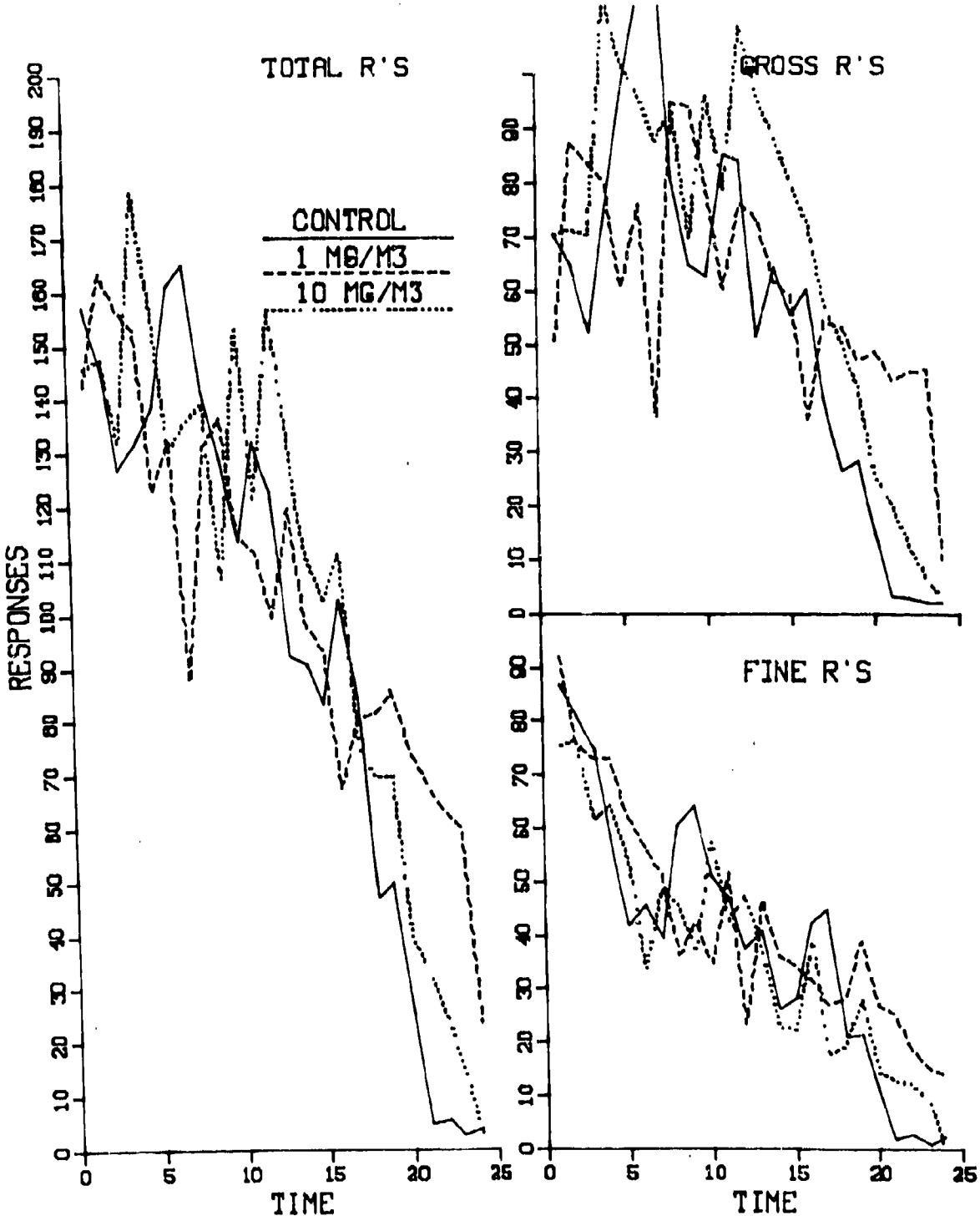

Figure 4. Female Rats, Spontaneous Activity Responses (Rs) Per Min, 65 Day (A) Brass Dust Exposure.

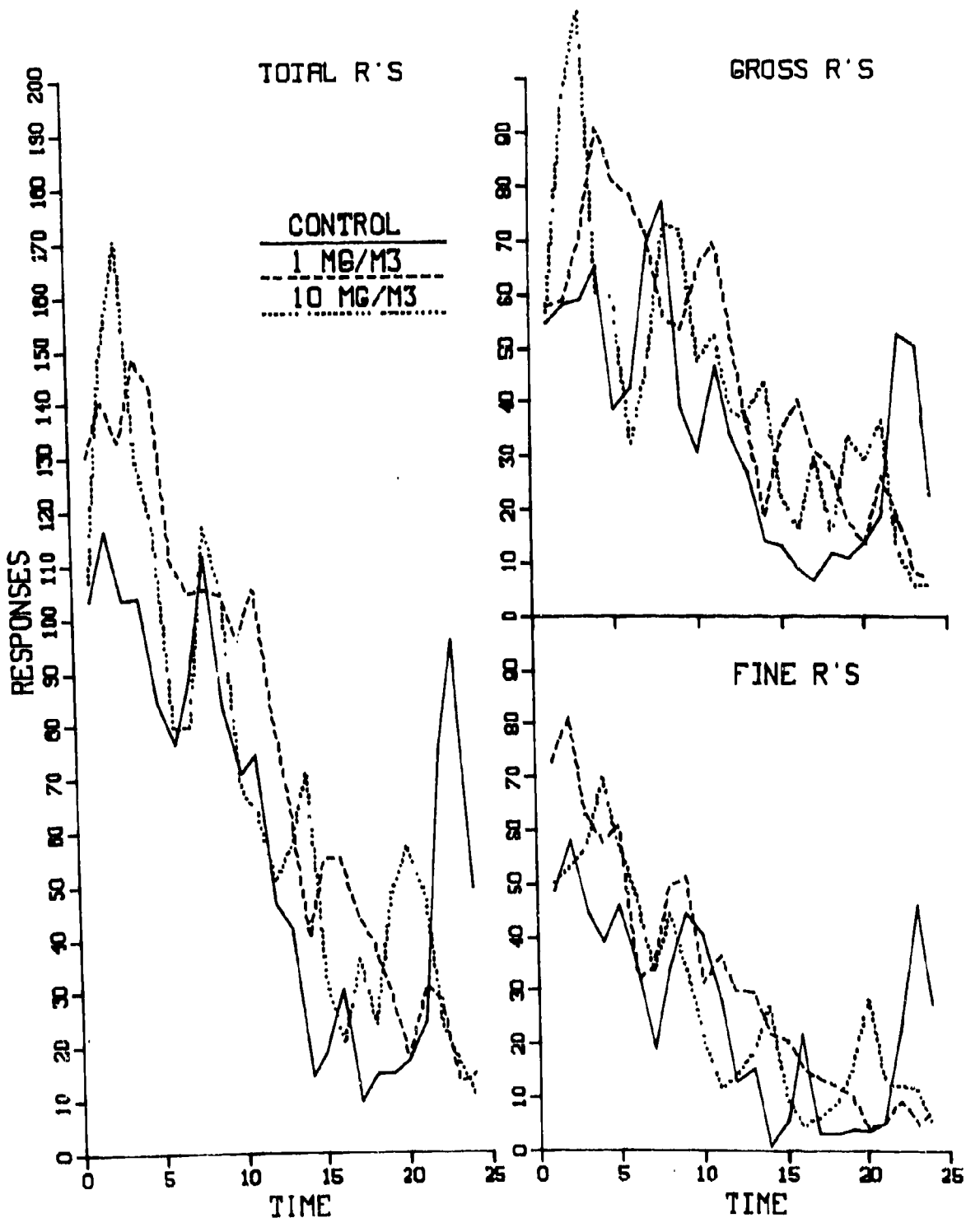

Figure 5. Male Rats, Spontaneous Activity Responses (Rs) Per Min, 65 Day (B) Brass Dust Exposure.

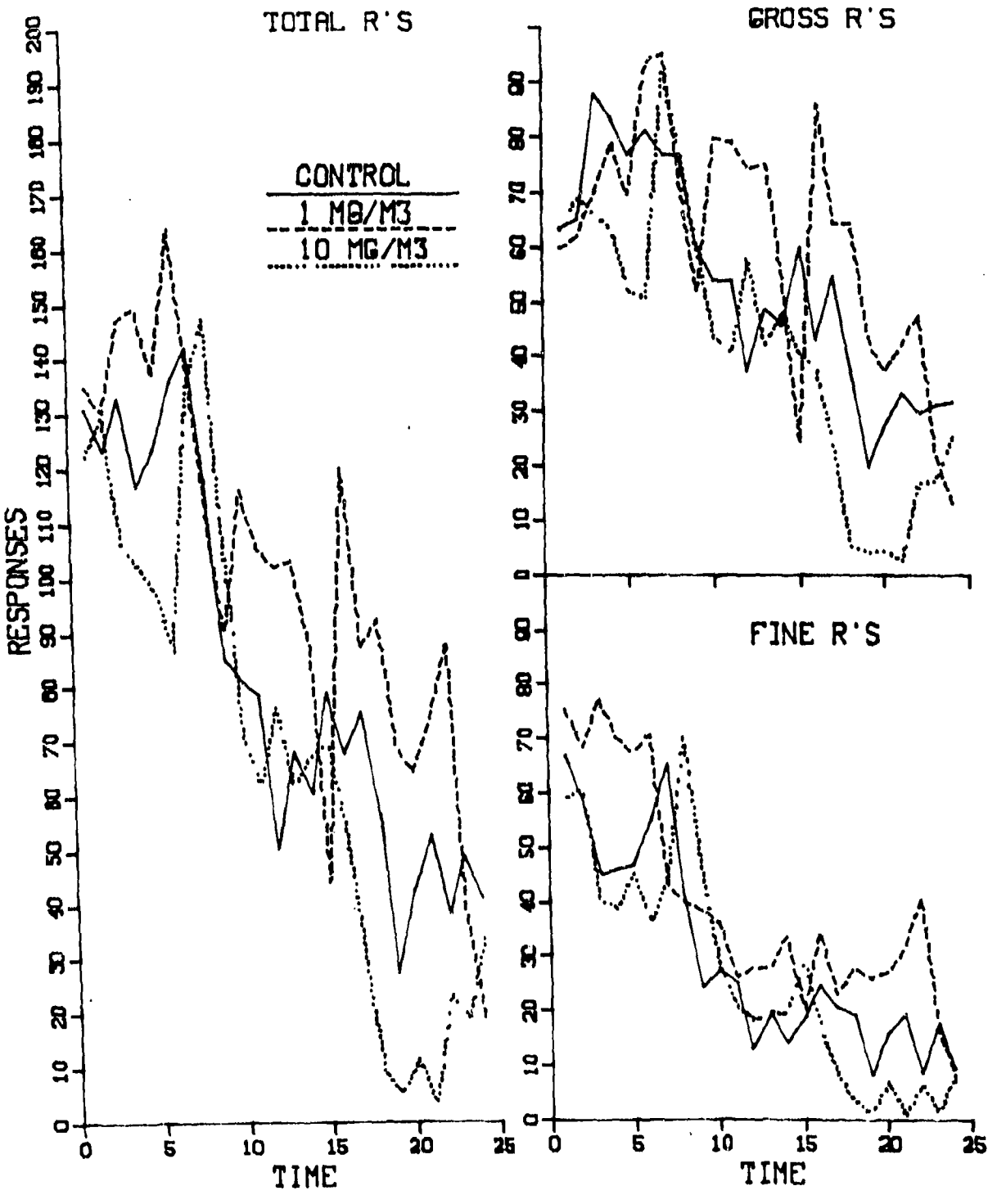

Figure 6. Female Rats, Spontaneous Activity Responses (Rs) Per Min, 65 Day (B) Brass Dust Exposure.

Table 2. Summary of Spontaneous Actvity Response Sensitization.

	30 A			65(A)			65(B)		
	Gross	Fine	Total	Gross	Fine	Total	Gross	Fine	Total
Male:									
Control	76 ± 7 [4]	102 ± 29 [2]	166 ± 17 [2]	80 ± 10 [3]	96 ± 24 [4]	144 ± 8 [3]	58 ± 15 [2]	77 ± 18 [8]	155 ± 17 [2]
1 mg/m^3	66 ± 16 [3]	91 ± 24 [7]	114 ± 28 [6]	71 ± 6 [1]	88 ± 11 [3]	143 ± 12 [1]	82 ± 10 [2]	91 ± 19 [4]	149 ± 20 [4]
10 mg/m^3	72 ± 7 [1]	77 ± 22 [9]	122 ± 22 [9]	66 ± 11 [1]	82 ± 14 [3]	142 ± 21 [1]	70 ± 17 [4]	114 ± 33 [3]	74 ± 30 [3]
Female:									
Control	74 ± 9 [5]	90 ± 19 [2]	156 ± 24 [2]	87 ± 17 [1]	126 ± 15 [7]	165 ± 20 [7]	67 ± 10 [1]	88 ± 11 [3]	142 ± 17 [7]
1 mg/m^3	72 ± 12 [4]	100 ± 17 [1]	156 ± 15 [1]	92 ± 8 [1]	95 ± 31 [8]	164 ± 10 [2]	77 ± 11 [3]	96 ± 14 [7]	164 ± 13 [6]
10 mg/m^3	74 ± 21 [1]	139 ± 16 [2]	200 ± 18 [2]	76 ± 6 [2]	115 ± 11 [4]	179 ± 6 [2]	70 ± 18 [8]	92 ± 14 [7]	147 ± 25 [8]

LEGEND: Data in the table are the mean peak activity (R's/Min ± SE) and the session time interval in which it occurred [minute]. There are no statistically significant treatment differences.

Table 3. Summary of Spontaneous Activity Response Habituation.

30 Day

	Total Slope (95% CL)	r^2	Gross Slope (95% CL)	r^2	Fine Slope (95% CL)	r^2
M-Control	-3.61 (±0.85)	0.78	-2.29 (±0.46)	0.83	-1.32 (±0.84)	0.33
1 Mg/M^3	-3.68 (±0.77)	0.71	-2.19 (±0.36)	0.88	-1.49 (±1.03)	0.29
10 Mg/M^3	-3.74 (±1.20)	0.65	-2.49 (±0.49)	0.83	-1.25 (±0.93)	0.26
F-Control	-2.53 (±2.07)	0.22	-1.61 (±0.80)	0.44	-0.92 (±1.44)	0.07
1 Mg/M^3	-6.39 (±1.22)*	0.84	-2.90 (±0.52)*	0.86	-3.44 (±0.94)*	0.07
10 Mg/M^3	-5.66 (±1.98)*	0.61	-2.19 (±0.86)	0.55	-3.48 (±1.32)*	0.57
65 Day (A)						
M-Control	-5.03 (±1.34)	0.73	-2.66 (±0.69)	0.74	-2.37 (±0.87)	0.59
1 Mg/M^3	-5.13 (±1.04)	0.83	-2.28 (±0.43)	0.84	-2.85 (±0.92)	0.65
10 Mg/M^3	-5.10 (±1.02)	0.83	-2.12 (±0.56)	0.74	-2.98 (±0.66)	0.80
F-Control	-7.13 (±1.26)	0.86	-3.21 (±0.63)	0.83	-3.92 (±1.34)	0.62
1 Mg/M^3	-4.60 (±0.87)*	0.84	-2.66 (±0.56)	0.81	-2.17 (±0.99)*	0.48
10 Mg/M^3	-6.40 (±1.42)	0.80	-2.80 (±0.50)	0.86	-3.60 (±1.34)	0.58
65 Day (B)						
M-Control	-3.44 (±1.69)	0.45	-1.58 (±0.87)	0.39	-1.86 (±1.06)	0.37
1 Mg/M^3	-6.22 (±0.70)*	0.94	-3.08 (±0.49)*	0.88	-3.14 (±0.72)*	0.79
10 Mg/M^3	-5.45 (±1.32)	0.77	-2.36 (±0.67)	0.71	-3.09 (±0.99)	0.65
F-Control	-4.74 (±1.00)	0.81	-2.24 (±0.59)	0.74	-2.49 (±0.67)	0.73
1 Mg/M^3	-4.46 (±1.21)	0.72	-2.44 (±0.68)	0.72	-2.02 (±1.06)	0.41
10 Mg/M^3	-5.57 (±1.27)	0.79	-2.59 (±0.61)	0.78	-2.98 (±0.84)	0.71

LEGEND: Data in the table are the regression coefficient (slope), 95 percent confidence limits (95% CL) and coefficient of determination (r^2) resulting from a least squares linear curve fit (HP-41C, Stat Pac) of session spontaneous activity generated at one minute intervals, for the three SA parameters and treatments. An asterisk (*) indicates significant difference from control at $p \leq 0.05$.

Table 4. Summary of Cumulative Behavioral Test Data and Body Weights.

Brass Dust Exposure Group

Sex: Treatment Group	Body Weight: Grams	Spontaneous Activity Test			Passive Avoidance Test		
		Gross R's	Fine R's	Total R's	PAR's	Shock Time: Min:	Shocks
30 Day Exposure							
Male:							
Control	277 (± 3)	1078 (±175)	1417 (±129)	2494 (±226)	8.0 (±4.2)	1.18 (±0.44)	8.7 (±2.6)
1 mg/m^3	286 (± 4)	928 (±215)	1254 (±138)	2182 (±285)	5.7 (±1.4)	1.49 (±0.26)	10.8 (±2.1)
10 mg/m^3	**246 (±10)	732 (±124)	1163 (±210)	1894 (±306)	4.8 (±1.4)	1.38 (±0.36)	11.0 (±3.2)
Female:							
Control	176 (± 3)	951 (±153)	1290 (±115)	2241 (±264)	9.3 (±3.5)	0.90 (±0.30)	6.7 (±1.8)
1 mg/m^3	174 (± 2)	686 (±131)	1074 (± 74)	1760 (±182)	11.8 (±2.3)	*2.10 (±0.52)	*16.0 (±4.1)
10 mg/m^3	168 (± 4)	806 (± 55)	1338 (± 92)	2143 (± 66)	8.4 (±1.7)	1.63 (±0.31)	*13.6 (±2.6)
65 Day Exposure							
Male:							
Control	299 (± 7)	857 (±131)	1047 (±215)	1903 (±321)	4.0 (±0.7)	1.27 (±0.22)	10.5 (±1.9)
1 mg/m^3	298 (±10)	733 (±135)	1001 (±164)	1734 (±243)	5.5 (±1.3)	1.82 (±0.43)	15.2 (±4.6)
10 mg/m^3	*270 (± 6)	469 (± 98)	775 (±149)	1244 (±242)	5.5 (±2.1)	1.59 (±0.43)	14.7 (±3.8)
Female:							
Control	174 (± 3)	930 (±126)	1329 (±162)	2258 (±276)	8.0 (±3.1)	1.10 (±0.30)	8.8 (±1.3)
1 mg/m^3	180 (± 2)	1011 (±129)	1458 (± 74)	2469 (±169)	10.5 (±2.1)	1.22 (±0.37)	9.0 (±2.6)
10 mg/m^3	**161 (± 4)	875 (±115)	1615 (±153)	2490 (±212)	9.8 (±4.5)	1.32 (±0.43)	9.8 (±3.4)

Table 4. Summary of Cumulative Behavioral Test Data and Body Weights (Continued).

Sex: Treatment Group	Body Weight: Grams M(±SE)	Spontaneous Activity Test: Gross R's	Fine R's	Total R's	Passive Avoidance Test: PAR's	Shock Time: Min:	Shocks
30 Day Post Exposure							
Male:							
Control	342 (±10)	607 (±122)	865 (±167)	1471 (±246)	5.4 (±1.2)	1.03 (±0.17)	8.0 (±2.1)
1 mg/m^3	321 (±10)	752 (± 94)	1081 (±159)	1833 (±241)	4.3 (±1.0)	1.16 (±0.15)	13.7 (±1.3)
10 mg/m^3	*297 (± 7)	650 (±147)	1035 (±196)	1684 (±324)	6.8 (±1.1)	1.19 (±0.29)	8.8 (±2.2)
Female:							
Control	180 (± 3)	708 (±112)	1281 (±268)	1989 (±352)	9.2 (±2.9)	1.41 (±0.38)	11.7 (±3.7)
1 mg/m^3	178 (± 5)	956 (±114)	1459 (± 81)	2415 (±186)	4.2 (±1.1)	0.61 (±0.16)	5.0 (±1.6)
10 mg/m^3	169 (± 4)	628 (± 70)	1003 (± 75)	1631 (± 61)	†10.3 (±1.9)	1.08 (±0.28)	6.7 (±1.4)

LEGEND: Listed in the table above for each exposure duration group are the treatment means and standard errors (M±SE) for behavioral data cumulated over a test session (SAT and PAT) and the pretest rat body weights. Statistically significant differences (ANOVA) at $P \leq 0.05$, from control (*) and 1 mg EA 5763/m^3 (†), are indicated.

recorded in the number of shocks delivered to those exposed at 1 and 10 mg/m^3 and an increase in shock time of those exposed at 1 mg/m^3.

3.3 Body Weight.

The treatment mean body weight data in Table 4 clearly indicate that only exposure to brass dust at 10 mg/m^3 resulted in a significant retardation in body weight gain. The effect was more severe in male rats and, in contrast to the females, was still evident 47 days post exposure (65B).

4. DISCUSSION

In general, there were no statistically significant dose-response effects of inhaled brass dust on either spontaneous activity or passive avoidance behavior of the rat. If it is assumed that a monotonic dose-related change (increase or decrease) in behavioral parameters would result from repeated daily exposure to brass dust, then the treatment data indicate that the air concentrations and exposure durations employed were relatively ineffective. However, if the behavioral response to repeated exposure-produced tolerance, was a U-shaped function or rapidly recovered postexposure, then it is possible that significant behavioral effects were missed by testing at inappropriate times.

Some behavioral responses to exposure did occur as indicated by the following observations:

- At the end of the first week, both male and female rats exposed at 10 mg/m^3 were found daily to be overtly hyperactive when observed after removal from the chamber. However, recovery from this effect consistently occurred overnight.[1]

- Two signs of pulmonary distress, hyperpnea and rales, became evident in these same rats during the fourth week of exposure and continued to be observed daily through the end of exposure.[1] The incidence of pulmonary rales remained relatively constant, while that of hyperpnea exhibited a bimodel distribution over time with peaks occurring during the sixth (95%) and tenth (75-80%) weeks of exposure. Both incidence peaks were preceded by weekly increases followed by a precipitous decrease; during the last week of exposure, the incidence of hyperpnea ranged between 2 and 8%. Hyperpnea (or dyspnea) in

animals, associated with the inhalation of a particulate like brass dust, is usually accompanied by a reduction in spontaneous activity directly proportional to the manifest severity of the sign.

The direction of residual dose-related changes in male SA, though not statistically significant, were consistently appropriate in each of the three sets of rats (30A, 65A, and 65B) for either a dose-related exposure or recovery effect, respectively. In view of the overt behavioral effects noted during exposure, the changes in cumulative SA of male rats most likely reflect residual recovery effects from the exposure-related hyperpnea.

In contrast to the males, the cumulative SA of female rats was inconsistently affected by brass dust exposure, actually indicating some increases after 65 days exposure (65A). Overall, the behavioral and body weight data, as well as pulmonary function and pathology, suggest that female rats were not as affected by brass dust exposure as the males.

The lack of significant exposure effects on the relative distribution of SA in time (i.e., sensitization and habituation) or on passive avoidance behavior, argue against any direct action of brass dust in the CNS.

5. SUMMARY

Male and female rats were exposed by the inhalation route to brass dust at 0, 1, or 10 mg/m^3, 6 hr/day, 5 days/wk for either 30 or 65 exposure days. At 14 days postexposure, the 30-day and one 65-day exposure duration group were behaviorally evaluated; a second 65-day group was evaluated 47 days post-exposure. There were no statistically significant brass dust dose-response or exposure duration effects on passive avoidance (step down) behavior or spontaneous activity. There was no evidence of any direct CNS effect in the rat related to the inhalation of brass dust. There was some residual evidence, in the session SA of male rats, of recovery from exposure-related hyperpnea.

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